3) —X666 1236

9
MAR 1955

New preps.

A. Harvest 200 ⊕/sml + plate MGA. Inc. from c. 11AM to 5PM, then to RT. room, 4°C.

B. 57 single cells planted. c 11AM to be followed microscopically. Examine at 3PM — variable size; mostly quiescent. Cells have grown mostly by enlargement. At 6PM, clone sizes again v. variable (some only 1 or 2, others ~1000). 3 clones picked as most numerous motiles. These were plated c. 6:30 PM. Unfortunately the MGA was floccy + plates could not be accurately interpreted. 1 may have had + well-marked trail. Expt. needs to be repeated.

Limited incubation allows swarms to be limited and counted.

A.

Harvest:	T	SW	Clusters:	1-Cells.	Total
100	2	1	8, 4, 3, 2⁶	65	~~77~~
10				4	4
20		2	2'	15	18
10		2		1	3
20	2		3'	15	~~18~~
20	1	2	2 3 3'	33	40
~~*~~ 10			2, 4', 5'	9	12
190	5	7	18	142	~~184~~ 172

but again rather poor lumpy and hard to score. Why so few trails here?

Conclusions: plating of selected clones may be promising method but needs to be repeated.

MAR 11 1955

MC8A (pipe ~~[illegible]~~ [illegible]) B ~~+ Tween. 0.2%~~ ~~[illegible]~~

Plated from inoculum cols. at 100/ml. Inc. ca 11-12 hours then R.T.

− Tween.

Est input	SW	T	C(2,3..)	1's	Total
40	2	3	$2^5\ 3^1$	35	46
20	2*	2	$2^5\ 3^2$	10	~~[illegible]~~ 21
25	3 **	0	$2^2 3^1$	14	20
85	7	5	$2^{12}\ 3^4$	59	8 ~~6~~ 7 ~~[illegible]~~.

* sw had 1' in center

+ Tween

Est input	SW	T	C(2,3..)	1's	Total
10	~~[illegible]~~ 3	0	2^4	2	9
10	0	1	2^1	3	5
20	1*	3	2^1	17	22
40	1*	2	$2^2 3^1$	26	32
20	$3^{\ddagger +}$	0	2^3	12	18
100	8	6	11 1	60	86

+ ~~[illegible]~~ Sw had 2 in center

‡ sw, at center had ○°○ and ⚥ nearby center.

Tween had no certain effect. how about survival?
but could be system. error of sampling?
Swarms were all about 1-2cm diam, somewhat variable even on 1 plate.
Note very low incidence of trails. Too early selection of ⊕?

86	~~[illegible]~~ 100
87	85

Should recover Fl$_a$ subs from A swarms: plate out residues of the drops for full test.

PA37 → SW666
selected clones
Effect of tween 80.

Misc. observations

1237

MAR 10 1955
MAR 10 1955

232

Prep. stab 3/9/55. (Quite fresh < 2 hours before incub.)

A. Freeze traps 11^0. Isolate to [232]. 50 by 11^47 AM.

(A–D) 2/row (a, b); 1–6. = 48 motiles. Incubate at 12^40.

B. Isolate 100 (+) to 12^40. To 2ml pen assay. Plate samples (≐ 40, 20, 10 cells) in MGA c/s Tween 80 .01%. Incubate frozen 1^05 PM — 11PM.

Examine ca 4^30 PM to select clones for plating. Isolate: (all have c. 10^2+ cells and:)

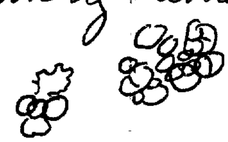
1 A3a	sw	Inc to 11PM then R.T.	sw. + 1's P hot. 10^30 AM.
2 B6a	sw		sw. + 1's shows 3 clusters (c. 2, 3, 4)
11 B3a	(20)		
4 B2b	(10)		sw. f 1's
5 C1a	(15)	Inc overnight RT	1's
6 C2a	(15)		1's
7 C4b	(10)		1's, 2'
8 C3b	(20)		1's
9 D2a	(10)		T
10 D3b	(10)		T
3 A5b			

as well as 10 more quiescent clones as controls.
(107/366; 11/200; 7/19; SW/COL)

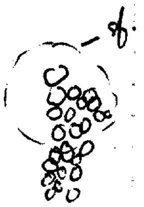
MAR 11 1955

A. Controls all had singles only. A3a, B2b, B6a all had swarms and colonies. A3a shows flares best: Isolate c̄ selection for later plating. The other two do so less strikingly. Arrangement should isolate the Fla⁻ sibs.

Among remaining plates, 5, 6, 8 show singles only. 7 has 1 2'

9: 

TRAILS IN 9, 10

10 

In selected groups 2T/7
unselected 0/10
hardly a dramatic result though warranting more extensive study.

Rexamn 232

1) A3a - act. swarm i residual Flg probable
4) B2b ~
2) B6a act. sw. ~ ~ ~
3) A5b also has numerous motiles (c. 20% or more) ~ swarms.

Otherdones are not unusable
(most have no evident Dan casual examination
some a few str.)

Try. B5a, hes ca. $10^4/10^4$
B6b $10^4/10^3$
~~B~~
C2a $/10^5$

MAR 12 1955
No trails.

MAR 12 1955 single clones from residual drops of clones A3a ~~B~~2b B6a and A5b are being diluted for motility and saved in selection. ~~also~~ also stab originals as 1237-A.

4/1: 1237 - 1A+, 1B+, 2+ } all b not i

MAR 12 1955

Isolates F4+ and − from 1-4.

Streaked on NSA, test on glc colonies on MGA ca 4-5 hours.

1 {1+A: 16−
  1+B: 17−} Compose original plating counts

3 3+: 29−

4 0+: 25−

2 7+: 27−

Later a second +

can be used for flare production
in unselected mostly lines
diff may depend on selective residue.

~~Picked 24.~~ pool +,− to stab.

Isolate 4+ by selection. (original mixed clones all also preserved for possible later need).

MAR 13 1955

2/11/47 1+A is still mixed.

MAR 1 2 1955

Bearings:

Since Jan 1, I have been primarily occupied with Salmonella trails. An important question has been the uniqueness of the"E particle". This would be hard to establish by quantitative data on the clones directly, and I have been principally concerned with looking at platings of small clones in motility ggar.
The results with SW-967 are not fully reliable owing to spontaneous "minor trails". This work has been done only since Febr. 23. Before that, from Jan. 11, I was mostly fiddling around. I must have been preoccupied with otherkinds of experiments too, or writing or what not, since relatively few experiments are recorded. There are also some experiments on crosses of heterogenotes, but DCG did most of the routine on these. From Jan-Febr., there were a number of misc, experiments on conditions of plating etc., which amounted to very little. There were some indications of major and minor trails. Also developed technique of trapping from conc. cell susp.

Summary of clone platings. (trails per clones/ per platings) and comments

			mass pla.
1227:	1/20/25	T: unique	
1228	3/9/10	T: majors unique, noticed addl minors	13/84/ 100?
1229	8/31/39	All T unique	24/198/200
		1229C: apont minors	
Total —x 967	12/60/74	All major trails unique. some dist. non-linear however. Confusion with spontaneous minors.	

—x SW966 Almost as prolific source of motiles

1232	4/16/25	Single majors, but other clusters. Clone size c. 2^6	27/159/202 + 1sw.
			53w/13/79/100
1233	1/34/47	Single small trails; a few other clusters. c. 26	
1234	6/15/48	Definite concurrence of smaller trails or larger clusters; hard to define. Av.Cl.S. c. 23-4.	30/149/200 3 sw. (not random)
1236	57 clones, follow micr/osc. plate only 3 NVG.		5/172 /200 18 cl., 7 sw.
1237 (eff. Tween)		This prepn. seemsng. although very fresh. Should compare directly with 1234 prepn.	11/ 173 /185 15 sw.

48 clones. followed micros. 4 gave swarms (segr. non mot.)
10 quiescent clones gave only singles; 6 with fairly numerous motiles gave 2 T's, + 1 with clusters.

MAR 1 2 1955

The principal point is perhaps best met by experiments like 1234, plating fairly early. A correlation of trails with pluricatenates like 1237 might be worthwhile, but more laboraious.

In view of sluggish motility of early log phase cells, this should perhaps be done with earlier clones in aged medium.

In some prelim. expts. yesterday, I noticed that TM2 transferred to aged medium supernate was more actively motile, particularly showing a more jerky motion with shorter free path. Examination of TM2 in motility agar suggests that many cells are directly immobilized, others move in apparent interstices, but still more slowly than normal. There must be a considerable accidental factor, and cell with numerous mobile progeny naturally has best chance to propagate a trail. Since genetically competent TM2 are immobilized, there can hardly be immediate correlation of genotype (or pluricatenxtation) and ability to move. Should watch trails in situ if possible. Why not?

Plans: continue with experiments like 1234. Compare this prepn. with 1237 in yield of trails. Continue with medium effects. Set up trails in situ, selecting cells which remain motile in gel. to see if these form the most chains.

→ x1140 n.s. why?

Do not forget many other carryovers:

EM
Gal, Nelson
heterogenote crosses
Hfr x F- !!!

G&C
DA Gal Salmonella.

DATE: REF:

a) 3/14/55 Collected ca (100) from 1234, 1236 preps for comparison + plated in MGA. (App. left at RT so result is misleading. Later brief incubation showed 1234: 6 swarms/87/(100)
1236: 35/68/97.
T not detected.

3/15/55

b) New prepn. 1238 (22 hours)
Fuse 1:40 Dool. (late) 3:20 – 3:50 A) 200/2ml ~~B) 50 ①'s but lost by error.~~

Running out of gelatin!

Plate out A samples. (Noted unreliable incidence of trails, might be related to quality of medium. agar was granular loose from reheatings!)

	Est. no.	C	T	Sw.	Cols.	Σ
SIC (B1)	100	2-3	82	5	4	95
	20	1	4	1	6	12
	20	11	4	1	3	18
	10	2	4		3	9
	10	3	3	1	4	11
α.	10					
	15	1	3	1	5	10
	185	21	100	9	25	155

See photographs. Note variability.

many swarms were centered c̄ C or T

Suggests variation of 1238C

Trails in (B1) particularly florescent.

1238
C-D
E

DATE: MAR 16 1955 ~~March~~ (Wed.) REF:

New suspn. (ca 9-11) Fuse drops 12^{30} to 3^{30} coll. (500) in 2.5 ml Plate, .25 ml samples

a). in MGA variously diluted. (Plating yesterday had shown remarkable incidence of large trails) Incubate to 4PM. Then R.T. refrig. (Plates 0 c. .25 ml). Figures indicate amount of NSB in this (-.25 ml). ~~½-1ml for samples.)~~

	NSB	Clusters	Cols.	T.	Sw.	Σ	
Est input 50 cells	0	2	53	2 (v. sh.)	2	59	
	1	2	55	1 "	0	58	
	2		59	2	3	64	Sw less compact
	5	5	46	6	0	57	
	7.5	18	39	11	3	71	sw diffuse
	10	11	19	19	3	52	" " ; TRAILS florescent!
Input (50)	0	1	93	1	8	107	
(100)	0		66	2	3		

Basic agar must have been unusually stiff. Swarms in NSB₀ v. compact also. Thus incidence and quality of trails increases with decreasing agar concentration!

---

also D) Fuse 12^{30} collect to 2^{30} D. P. drops to 3PM Incubate to ? 5^{30}PM. (Some refrig. E) to 1215 PM Thurs for plating.

Isolated 12:15 Thurs.

C: 13 blank 4 contam) movies cols. 1 swarm + 18 clones } all except C1 singles only

C1 has 1 major (v. prof.) trail, 6 moderate 40, 5 clusters + 20 clusters

D 8 blank, 5 clones.

No shot - owing to contaminant also.

1238C1 – nepheto.

T_1 c. 400 ohms

$T_{...}$ << 50

DATE: Mar 17, 1955. Thurs REF:

1	2	3	4	5	6	7	8	9	10

Met Kornberg visit later in PM. ca (1000)/ml cool. but no used since 1240 prep. wasfreshn next day.

1238 prep. Fusetraps 12N. Spottedout 1:40 Clonespiked and incubated c. 2^30 – 6PM (3½ hours). Plate in MGA ± diluent USB. (c. 10ml: 15 MGA). Also noted that these dessiminated cells showed internal structure (nuclear?)

MGA standard still .4% Agar 8% Gelatin

A). MGA stragt. | 1 contam 12 blanks 16 clonesadd. | 2 swarmed < No def. colonies; 2 large plaques; " 0 " (vagrescent initials; 1 plaque

. all singles. <u>no trails</u>

B) MGA 60% | 1 swarm – 7 plaques 4 single cols. 8 blanks 8 clonesadd.

1. all singles
2. " "
3.
4. " "
5. " "
6. at least 11 trails, 1 major. * Sa photog. > 150 cols! / T_2 c. 30
7. all singles

Total 48.

3/22/55

Counts on motility plates:

1239A:

11	103
61	140
144	67
119	41
17	181
37	115
54	92
196	51

1239B:

97
225
60
134
100
102

1238D:

22	39
36	25
40	6
15	44
52	6
6	61
13	13
9	18
44	39

Plates marked "C"

9
9
2
31

DATE: March 19, 1937 Fri REF:

Tool (1000)/ml. and plate .1ml, .2ml samples. Inc c. 2–11 PM then RT

1. MGA (100). 4 swarms, 56 singles (remnants of yesterday's MGA; Ref. MGA equally diff.)
12 2's
8 3's
5 short tails c 6 clusters

? 2 MGA (200) (somewhat looser – cf. swarms) 6 swarms 23 Trails ext.
33 singles 4 2's 7 3's 6 clusters

3. MGA (200) [firm!) 4 compact swarms 2 or 3 short trails, rest singles
dilute 40%

4 MGA Wilson trails nebulous, swarms limited 47 trails 3 sw (1 cT) 6 clusters
26 singles
5 3's

5 MA (4.5%) 3 sw: 1 T, 1 center ;

| cc6 mils
incubate 3:15 6 6 cc

Out of gelatin! Use MA (.45% agar) (a) and Wilson gelatin (n.g) (b)

two groups: (a). 9 plates (2 large) are blank ; 19 plates (6 large) have clones

a) (5) ① covered by a swarm ; 3 zones of lysis. In addition ca. 18
all trails of some extent, some considerable. May be hard to photograph.

19 clones

photo ② 7 + c^10, c^9, c^4 + pyramidal cluster, &50.

3. 7 + c^2, c^6, c^4

4 12 + c^6, c^6, c^4 ; c^9, c^12, c^7, (a wT ca 102), c^9, c^6

⑤ 32 + (T+), + c^7, c^8, c^6, c^3, c^11, c^2, c^4, c^6, c^7

also (14) 51 (1c?), 22 (1c), 9 + 2c [sketch sketch], 63, 94, 18 + [sketch] ;
15 [sketch] ; 23 ; 16 + [sketch] ; 33 ; 13 (incl 2'), 60, 11, 14;

(Gen)

b) mill, wash gelatin again. (a sloppy - gelatin must bind again!). 9 blanks 10 clones.

	1.	5 short tails	+ 9 cols
*	2.	13 prol. tails	+ 4 cols.
	3.	4 short "	7 cols.
	(4)	12 prol.	7 cols
	5	2 mod. "	23 cols
	6	14 tails	11 cols
	7	1 good t.	+ 8 cols.
	8	12 " t.	18 cols
	9	1 linear t	15 cols
	(10.)		
	~~11~~.	7 tails	. 0 cols!

these tails off. linear

Conclusions 1938-40.

among motile initials plated

1. Incidence of trails/varies with the fluidity of agar. Addition of 40% diluent gives very high incidence. In any event, agar that is hardening tends to be quite inhomogeneous, if maintained at critical temperature.

2. Single clones can give at least one trail + large clusters in harder agar, and numerous trails in softer. This is clairly an unreliable criterion for singularity of catenation of higher order.

Further plans:

1. A few more tests of fluidity and related variables for photographic documentation.

2. Shift studies to direct pedigrees; need some further data on irritants; inh. of cell size growth and chemotactics.

3. EM transfers.

4. Write it up!

Butterfield Doubtless spent this week cleaning up around lab.

What happened last week? N.G.

① Acetyl gelatin

② No guiding what to do next on chains!

③ Change medium < D(o) - flattens out too far (wets glass - how counteract?)
necessary 10% - poor growth.

Acet-serum in H_2O seemed limited. Metal poisoning? - Try pure water.

problem: don't want to follow mid. pedigree more than 3-4 generations but minimum size drops down too many cells ~~at~~ ultimately. Should have 1. inal clones of about 300 cells. Try partly exhausted medium.

④ Serum effect

i / TML of course diff. owing to H_2 1, 2

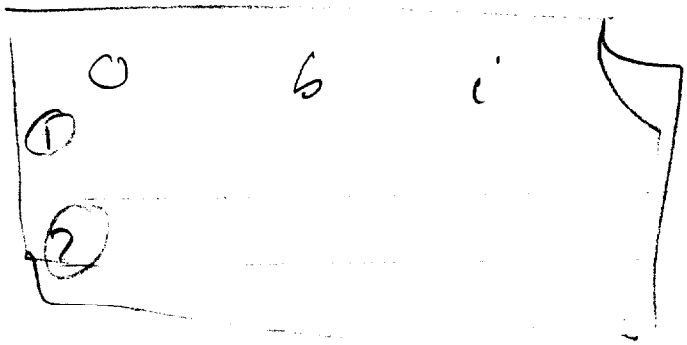
b < 1237-2(H_1 b). at first almost completely inhibited, but some probably inhibits by anti i at 1:100. with overnight growth, see active motility and agglutinated clumps - May still be worth trying at 1:100. (serum titer ca 10⁴).

APR 1 1955

Semm effects

① 1237-2 = b ② TM2 = i

1. Try against sums 1/100 in both.



0 cells almost completely ruled by b sems, partly by i

i cells partly ruled by i or b (1,2 comps?)

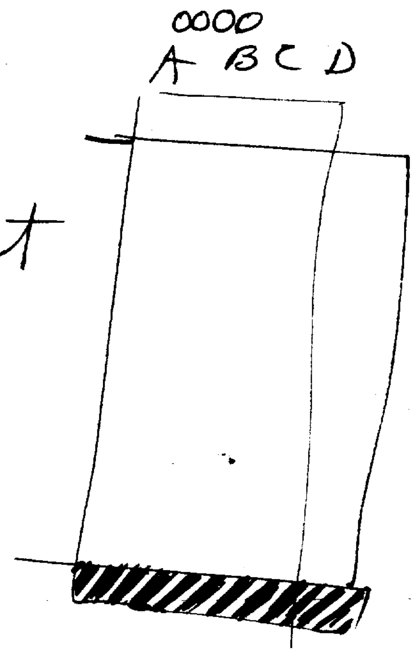
Plumb
Thames
Co. Eff

37-X866

1241 (E)
MAR 31 1955

Inc. Centr Decant.
New preps. 1/2 : 1/2 10AM – 11:45 – 12^10 Refr.

E F
Collect in 10% broth ; D(0).



[illegible] 2^45 (31) by 2^55 spot at
E) 22 deposited to 3^05 A↓, B.

F (35) to 3^15 Spot at D, C, C'
to 3^25
Stet R.T.

E: only 1-4 cells saw. mostly (1)
~~5^30~~ ; 7^30 – 8^30
F 1 – 4 (often (2)) far more active!
spreads [illegible] easily.

Viscosity?

9^30 A1 Poisoning to [illegible]; very little growth.

→ Sc866

1241

Misc.

3/28 NG

3/29. 3) 10% broth dilution. A) Penassay 40 ①'s, same split, 1-4. No staining many chained, not closely examined (too many cells, >10⁴/.) cut 3 swarm-clones A < ⊕ 1 ⊕ 2 C / ⊕.

save for later check of identity + homogeneity

B < ⊕ 1 ⊕ 2

3/30 cf. 10%, 100% Penassay: middle tubes

F 12/58

DATE: APR 5 1955 REF:

New prepns. a): from aerated SW-666, b) from unaerated culture.
Both + 1:1 FA-37 10:20- 11:35-12:10 Refr. (Incub. in rot.)

Note: Rotator now standard for aeration.

A): Prepn. a). Fuse traps 12:30, collect ca. 100 motiles, but use to spot (2-2:15)
DCG pick to 3:30. Incub. in .5 ml Penassay to c. 6:15 PM. Plate in MGA +
40% NSA as diluent.

B): Same collection, plant in spent broth (SW666 Aer.) to cf. total clone size. St. RT.

C): Prepn. b) Fuse 4 PM. Collect 5:30-6:00 (some needle tr.) This prepn at least as good as a, probably better.

1000 (+) / 1 ml. Plate 0.1 ml samples in large Plates; .05 in small. Compare MGA, + 40% NSA dil., + 60% NSA dil. Incub. 37° c. 6:45 - 11PM/ Then RT to P6; then refr. for analysis. (dil. MGA very soupy!)

APR 6 1955
Hold A,C for study.

(Spent broth = overnight SW666A, 60° 20 mins., the sediment and decant. Numerous fine granules still present).

B

11:15 -12:15 AM Examine B). Note that clones are limited to 100-1000, while Penassay gives at least 10x as many. 18 clones (in spent broth) examined: (sequence not retained).

3 - 0's 2: about 100 Fla⁻. 5 had 1+/c. 100, of which 4 isolated to broth for plating clones. 3 (4?) had 2+ (+ Cl, 2,3), each isol.

C14 had 29+/1000 C13 23/1000 C6 12/-00. (These picked collected and plated without further growth.

C5 had 10+/-00. Plant individually, pick to broth for clones. (C5-1-2 maybe 2/0 instead of 1/1).

(Inc. 12:30 - 3:00 PM. Plate in MGA-40).

APR 6 1955

DATE: REF:

Note: OCG picked in sequence, but this was randomized for plating. Seems cold

#'s are empty:

(18) 1, 2, 5, 6, 8, 11, 12, 14, 23, 24, 25, 28, 31, 35, 41, 44, 45, 46.

note: 6n+1, 6n+3 ($n = 0-7$) were MGA all others MGA + 40% WSB.

(1, 7, 13, 19, 25, 31, 37, 43
3, 9, 15, 21, 27, 33, 39, 45)

swarms (or cart): 9 (plague c. 50 1's); 13 (c. 4 1's opp. ca 4 ⊕'s)

26: + c. 10 colonies, mil. 5-6 trails

29 + c. 20 trails, few 1's.

(7) 33: patchy lysis, swarm + 3 3's, 11'.

36: Prob cart.; No colonies — cart.

39 sw + c. 100 singles + short chains.

MGA:

3 ca 60 no T 2 2's.

7 6 1's

15 c. 60 1's

19 c. 45 1's,

21 6 1's 8 c's (3-6) 1 T35 1 T10 (closely).

27 Imagi trail + 9 c's (3-7) + 6 1-2's.

37 1 T ;8 1's 3 2-3's.

43 111's 2 2's

MGA 60% 40% WSB

4. ca 20-30 profuse trails ~~sw~~ 50 1's. somewhat detached

10 >100 all 1's

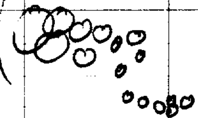
(11) save for plot. { 24, 10, 24, 23, 14, 6, 15, 10, 19, 24, 30, 47, 13, 11, 100, 150, 6, [illegible], 23, [illegible], 14, 15, 16 and others. (colonies per trail.) 37-1's 5 c (<10) 21 T's.

18 9 T's; 38 1's

18 seems somewhat tighter agg, 12 T; 3 clusters (3-7); 38 1's

DATE: REF:

1	2	3	4	5	6	7	8	9	10

20 1T () ; 60± 1's

22 (partly slopped) Profuse trails about 1:3 1's

(30) Profuse trails, 16T, 5C (3-5), 551's {T's include c.80, 80}

32 " " 7T, 1C, 561's.

34 45 1's

38 17T's 3C's 45 1's

40 > 500/plate ; some prob. trails but too crowded to count.

42 14T's 33 1's (rough counts).

save for photos 11, 18, 30.

Test "swarmers" in B tac in order
9, 13, 24, 29, 33, (37) X, 39 others ✓

APR 9 1955

{Note BAADS letter — #11 illustrates clone c̄ profuse trails. But this was incubated 15 hours. of "mcp trails"} (Usually no progression over c̄. 8 hours but must be controlled! Need check on trail progression at R.T.)

37 → x 666

1242 + B

APR 5 1955

1:1

① New prep. A flask aerated SW666 x — FA37 } ~~[illegible]~~ Rotate 10^{20} to 11^{35}.
B non aerated " x —

(Rotator now in op'n and generally used for aeration rather than bubbling)

10^{20} - 11^{35} - 12^{10} Refr.

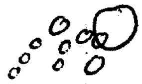
②. Freeze drops A - 12^{30}

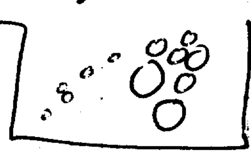
see 1242

APR 7 1955 9^{30} AM Score series C.

Note: many plates have small swarms, doubtless contaminants. Save for characterization & cf. 1243 A.

C1 2 plates. A 10^2 1's; no clear T. 1 fuzzy stag of 8 mm glass. B. c. 60 1's

C2 A 60 1's 1? T (fuzzy: ) } terminal chains?
B ditto



C3' A. c 40 v.s. 1's & B the same — many cont? scr.

C3 a " "
b " "
c " " Heavy contam. } N.G.

(824)

C – ~30 1's no T

C – 0

C – ~40 1's. no T.

Terminal ⊕ have poor chance of starting a longish trail.
Medium OK.

~25 – 1 } 20 small 1's + cut swarm
2 } ditto
3 " "
4. " "
5 40 1's " "
6 " " " "
7 " "
8 0 " "
9 30 1's " "
10 30 1's " "

(small angles prob. also cut.

5/10 plants → clones. all of these had presumably petered out & gave no trails at this point.
cf. 6, 13, 14

1242B

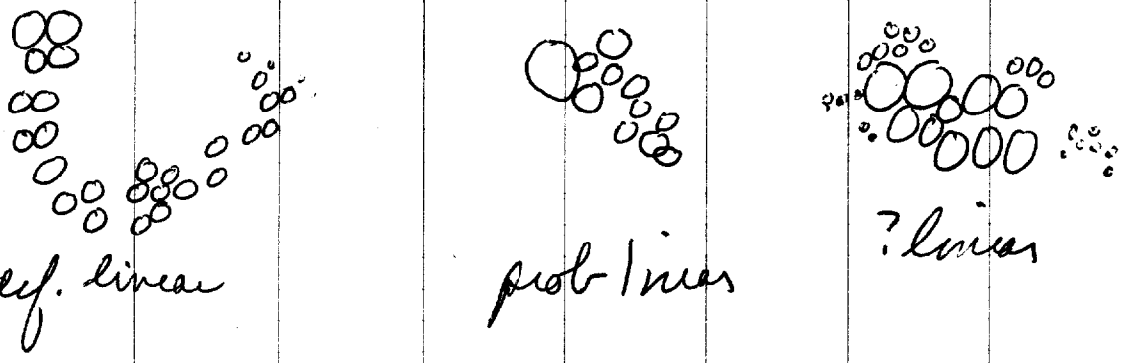
DATE: APR 7 1955 REF:

1 2 3 4 5 6 7 8 9 10

B6: 7 1's 6 clusters 3 trails

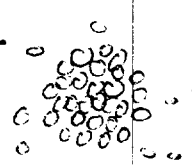
Σ 16

Input:



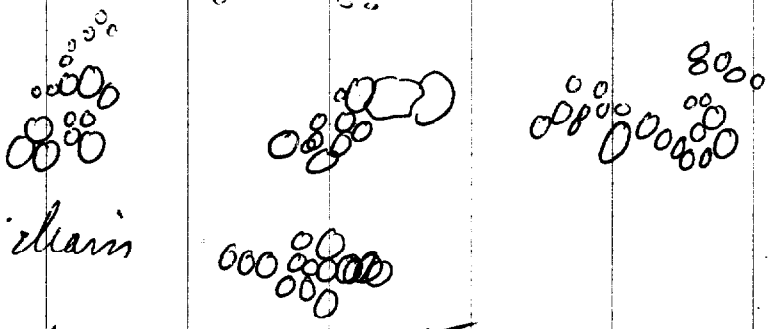
def. linear prob linear ? linear

B13 16 1's 1 globular cluster



3 linear clusters (4 B);

and



Σ 25

prob uni'dians

to total 16 1's, 8 "trails", 1 cluster

B14 See photog. Most trails linear in esput.

21 1's 12 T's 5 C.

But not C13 nebula!

DATE: APR 6 1955 REF:

	1	2	3	4	5	6	7	8	9	10

3:40–6 PM Trap 1000 D / 1 m². Plate 0.1 ml sample 25 ml total volume.

				T	C	I's	SW	Σ
1	MGA			88	12	9	5	114
2	MGA		0.03 ml					
3	10 ml	Penassay		129	0	8	12	149
4	"	H₂O						
5	"	Saline						
10	"	NSB						
7	5 "	NSB	misinoculated?					
8	7.5 "	"						
9	12.5 "	"						
10	15 "	"						

3 gave best development of trails. Also routinely from worn os. Layers evidently too shallow for extreme gas.

Note: this batch of MGA already showed deposit of gelled agar and was probably inordinately soft to start with.

4-5-6 agar is showing almost 100% trails! all with photo's St. room trip to bring out swarms more sharply.

This expt. n.v.g. for comparison of agar density owing to looseness of original MGA.

DATE: APR 8 1955 REF:

	1	2	3	4	5	6	7
		i's	i's	T's	SED	Σ	
* 1				3+		68	
				11		64	
				1		54	
			2^2	0		20	
* 2				1		43	
			1^{2-3}	0		78	
				0		62	
				2 linear		43	
3				0		59	
			3	0		67	
4				2 v.s		12	
				4 s.		20	
5						0	·
						0	·
6	0					0	·
				8 short		46	
7				0		49	
				0		47	
8				1 sh.		82	
				2 sh.		65	
9						0	·
						0	·
10				11 short		65	
						0	·
11			✓	0		62	
			✓	0		0	··
12				0		0	·
			1^3	0		44	
13				0		0	·
				0		6	
14				1		20	
				1 extens.		17	
15						0	·
						0	·
16				6 m.s. - linear		53	
				5 " ; 1 glob.		41	
17						0	·
			1^2			24	

Inc. 5:30–11 PM, then RT. Scored 9 A 8. This dil. MGA may be stiff

Owing to probable stiffness of agar, expt is inconclusive.

1-2 prob. worth photographing.

Photography 1244'

APR 8

1243-3 DATE: (Lt. value 13-25) REF:

1	2	3	4	5	6	7	8	9	10

for calib. f6.3 - f9 at 1/20 +X film found best (as indicated by light meter!) for large plates, on light box. Leica set-up

no lens for large. c [illegible] in front. Develop D19 - 6 mins.

Use 1242 ~~#3~~ #1-6
#3 = sample.

f8 1/20 +X.

1243 1-6. f8.
1 = #1 c 1½ secs.
2 = #1 1/20 "
3
4
5
6 etc.

Same stops

EMB large plate (margin etc. for timing?) 1/20 f4.5

1242C -0
-0
-40
-40
-60
-60

1238 B1 marked.

small plates ~~same~~ with lens #3, no ext tube.
1238C1 ...
1244A1 (4), A2 (4), (marked).
1242 C13

4, 9. weary solutions!

1244'

DATE: 4-9. APR 9 1955 REF:

1	2	3	4	5	6	7	8	9	10

Photography 35mm Leica astrograms for yesterday
Large plates ~~at~~ no front lens.

(3)(4)(5)(6)(7)(8)(9)

1243 1-6 1243 : 3, 4, 5, 6, 1, 2 f8 1/20 sec.

7 λ-6A6

7

HF 7-6

ag came out beautifully
D19 clear 8+ minutes

EMB f3.5 2744-x last + 2769 EMB overexposed

1426A + 6.

~~1243-6 again~~

1238B1

1242C -0
-0
-40
-40
-60
-60

small plates #1 3 lens. 1241-1 (4)
-2 (4)

1242A 11, 30, 18

1242 C6, C13, C14

1231B

Resume 1242 - 44.

APR 9 1955

1242

A) clones from single initials.

18 empty

7 had swarms (diffused) + trails [one contain.)

8 clones on MGA standard: some T's, usually poorly developed.

13 " " MGA 60%.

*photographed.

* 20 T; 50 1's	100 1's
9 T 38 1	75 1's
* 12 T 3C 38 1	> 500 1's, T's.
1 T 60 1	
> T	
* 16 T 5C 55 1's	
#(32) 7 T 1C 58 1's	
17 T 3C 45 1	
14 T 33 1.	
*#11 21 T 5C 37 1's (count complexity of trails).	

Terminals

B) Plant in spent broth 18 planted (17?). Looks for F/a+ /c 10^3 F/a-

3 0's. 3 0+ 5 1+ 3 2+ and

groups of 29, 23, 12 (C6) (C13) (C14) from 3 others. Plant these as groups or clone in MGA60. From singles, No clear trails from clones. Groups:

~~C13~~ C6, 13, 14 See photos. { 3T 6C 71 ; 1T++ 8T 161 ; not counted }

Nothing at all!

APR 9 1955

ctd.

1242C Platings in MGA, 60%, 40%. in small and large plates. Large plates: Swarms rather messy but photographed. MGA-0 showed compact sw, no T. 40, 60 about equivalent development of trails

Small plates ~~not yet studied~~ equally messy, suggest that MGA40 is sufficiently dilute to bring out most trails; more profuse at MGA60.

1243. Is simply group initials, various media. See pictures Pernassay or Bay as diluent. (1-6).

1244. (A) Like 1243 but excess nonmotiles interfered. MGA standard rather stiff; MGA 40-50 optimal

(B) Sib clones. Agar probably too stiff but photogr. sequence 1-4, 2-4.

New notes on Bruce - { abstract together? or MOB } c. 4/10/55 -

① my cells don't get stuck
② they stay motile - usually both n_1's are ⊕
③ don't like "replicas" of mine.

"We have never obs. E cells in > 1 sublin ~~[limited observations]~~". How many E clones have been similar

B claims one case of E at n_{22} only 1?

Need my own data on [E+E] or [E+S] in 1 clone

style; numerical calculations; first conclusions first.

Where are pedigrees?

No time now to clean up pedigrees.